

2008

Application No.: 10/680,963  
Amendment Date: September 15,

Reply to Office Action of: 28 May 2008

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AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Withdrawn – currently amended): A process of making a human-like glycoprotein in a yeast host cell which is has been genetically engineered to be diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and includes to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity comprising the step of introducing into the cell a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity.

Claim 2 (Withdrawn – currently amended): A process of making a human-like glycoprotein in a yeast host cell which is has been genetically engineered to be diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and includes to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity comprising the step of expressing in the cell a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity.

Claim 3 (Withdrawn – currently amended): A process of making a human-like glycoprotein in a yeast host cell which is has been genetically engineered to be diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and includes to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity comprising the step of expressing in the cell a nucleic acid molecules encoding one or more enzymatic activities that produce *N*-glycans comprising GlcNAc<sub>3</sub>Man<sub>3</sub>GlcNAc<sub>2</sub>, GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> or GlcNAc<sub>2</sub>Man<sub>5</sub>GlcNAc<sub>2</sub> bisected structures.

Claim 4 (Withdrawn – previously presented): The process of claims 1 or 2, wherein the *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity produces a bisected glycan.

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**Claim 5 (Withdrawn):** The process of claims 1 or 2, wherein the glycoprotein comprises a bisected glycan.

**Claim 6 (Withdrawn – previously presented):** The process of claims 1 or 2, wherein the activity is intracellular.

**Claim 7 (Withdrawn):** The process of claims 1, 2, or 3, further comprising the step of isolating the glycoprotein from the host cell.

**Claim 8 (Withdrawn – previously presented):** The process of claims 1, 2, or 3, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., and *Candida albicans*.

**Claim 9 (Withdrawn):** The process of claim 8, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, and *Pichia* sp..

**Claim 10 (Withdrawn):** The process of claim 9, wherein the host cell is *Pichia pastoris*.

**Claim 11 (Withdrawn):** The process of claims 1, 2, or 3, wherein the glycoprotein is a therapeutic protein.

**Claim 12 (Withdrawn):** The process of claim 11, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor  $\alpha$ -chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin,  $\alpha$ -1-antitrypsin,  $\alpha$ -feto protein, and DNase II.

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## Claims 13-75 (Cancelled)

Claim 76 (Currently amended): A yeast host cell which is has been genetically engineered to be diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase and includes to include at least an α-1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity comprising a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity.

Claim 77 (Previously presented): The host cell of claim 76, wherein the catalytic activity is intracellular.

Claim 78 (Currently amended): The host cell of claim 76, wherein the cell further includes a nucleic acid molecule encoding a mannosidase II produces *N*-glycans comprising GlcNAc<sub>2</sub>Man<sub>2</sub>GlcNAc<sub>2</sub> structures that are capable of reacting with the GnT III catalytic activity.

Claim 79 (Previously presented): The host cell of claim 76, wherein the *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity produces a bisected glycan.

Claim 80 (Previously presented): The host cell of claim 76, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia kochlamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., and *Candida albicans*.

Claim 81 (Currently amended): A yeast host cell which is has been genetically engineered to be diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase and includes to include at least an α-1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity comprising a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase II (GnT II) catalytic activity and a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity.

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Claim 82 (Previously presented): The host cell of claim 81, wherein the catalytic activity is intracellular.

Claim 83 (Currently amended): The host cell of claim 81, wherein the cell further includes a nucleic acid molecule encoding a mannosidase II ~~produces N-glycans comprising GleNAcMan<sub>3</sub>GleNAc<sub>2</sub>-structures that are capable of reacting with the GnT III catalytic activity.~~

Claim 84 (Previously presented): The host cell of claim 81, wherein the *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity produces a bisected glycan.

Claim 85 (Previously presented): The host cell of claim 81, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., and *Candida albicans*.

Claim 86 (Currently amended): A yeast host cell which is has been genetically engineered to be diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and includes to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity comprising a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity and a nucleic acid molecule encoding a mannosidase II catalytic activity.

Claim 87 (Currently amended) The host cell of claim 86, further comprising a nucleic acid molecule encoding an *N*-acetylglucosaminyltransferase II (GnT II) catalytic activity.

Claim 88 (Previously presented) The host cell of claim 76 that is deficient in an *OCHI* mannosyltransferase activity.

Claim 89 (Previously presented) The host cell of claim 81 that is deficient in an *OCHI* mannosyltransferase activity.

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**Claim 90 (Previously presented)** The host cell of claim 86 that is deficient in an *OCHI* mannosyltransferase activity.

**Claim 91 (Previously presented)** The host cell of claim 76 that is deficient in the Dol-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-Dol mannosyltransferase activity.

**Claim 92 (Previously presented)** The host cell of claim 81 that is deficient in the Dol-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-Dol mannosyltransferase activity.

**Claim 93 (Previously presented)** The host cell of claim 86 that is deficient in the Dol-P-Man:Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-Dol mannosyltransferase activity.

**Claim 94 (Previously presented)** The host cell of claim 76, further comprising a UDP-GlcNAc transporter.

**Claim 95 (Previously presented)** The host cell of claim 81, further comprising a UDP-GlcNAc transporter.

**Claim 96 (Previously presented)** The host cell of claim 86, further comprising a UDP-GlcNAc transporter.

**Claim 97 (New)** The host cell of claim 76, wherein the yeast is a methylotrophic yeast.

**Claim 98 (New)** The host cell of claim 81 wherein the yeast is a methylotrophic yeast.

**Claim 99 (New)** The host cell of claim 86, wherein the yeast is a methylotrophic yeast.